

SEQUENCE LISTING

<110> MILLER, JEFFREY C.
 ZHANG, LEI

<120> METHODS AND COMPOSITIONS FOR TARGETED CLEAVAGE AND RECOMBINATION

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<140> 10/587,723
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 <151> 2004-02-05

<150> 60/556,831
 <151> 2004-03-26

<150> 60/575,919
 <151> 2004-06-01

<150> 10/912,932
 <151> 2004-08-06

<160> 212

<170> PatentIn version 3.3

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 <223> Target sequence for the hSMC1-specific ZFP

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 <222> (23)..(34)
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<223> human x chromosome SMC1 region

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<223> an amplification product derived from a mutated hSMC1 gene

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ggtcgcgagg agcaagtgtt tggtgttgcc atgcaacaag aaaagggggc ggaggcacca 180
cgccagtcgt cagctcgctc ctcgtatacg caacatcagt ccccgcccct ggtcccactc 240

ctgccggaag gcgaagatcc cgtaggcct ggacgtatt tcgcgacatt tgccggtcgc 300
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 <223> Target sequence for the second pair of IL2Rgamma-specific ZFP

<220>
 <221> misc_feature
 <222> (23)..(34)
 <223> Target sequence for the second pair of IL2Rgamma-specific ZFP

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<400> 8

Tyr Lys Asn Ser Asp Asn Asp Lys Val
 1 5

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<220>
 <223> IL2Rgamma gene

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<212> PRT
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<220>
<223> IL2Rgamma gene

<400> 10

Tyr Lys Asn Ser Asp Asn Asp Lys Val
1 5

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<211> 624
<212> DNA
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<220>
<223> mutated IL2Rgamma gene

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gcaaaaaaag gagatccacc tctaccaaac atttgttggt cagctccagg acccacggga 180
accaggaga caggccacac agatgctaaa actgcagaat ctgggtaatt tggaaagaaa 240
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acatatctcc agtgatcccc tgggctccag agaacctaac acttcacaaa ctgagtgaat 480
cccagctaga actgaactgg aacaacagat tcttgaacca ctgtttggag cacttggtgc 540
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attacaattc actggccgtc gttt 624

<210> 13
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<220>
 <223> human beta-globin gene

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 actcctaagc cagtgccaga agagccaagg acaggtacgg ctgtcatcac ttagacctca 120
 ccctgtggag ccacacccta gggttggcca atctactccc aggagcaggg agggcaggag 180
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 <223> an amplification product derived from a mutated beta-globin gene

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 agtagcaatt tgtactgatg gtatggggcc aagagatata tcttagaggg agggctgagg 120
 gtttgaagtc caactcctaa gccagtcca gaagagccaa ggacaggtag ggctgtcatc 180
 acttagacct caccctgtgg agccacaccc taggggtggc caatctactc ccaggagcag 240
 ggagggcagg agccagggtc gggcataaaa gtcagggcag agccatctat tgcttacatt 300
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<220>
 <223> IL-2Rgamma gene

<220>
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 <223> target sequences for the 5-10 ZFP/FokI fusion protein

<220>
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 <222> (31)..(42)
 <223> target sequences for the 5-8 ZFP/FokI fusion protein

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42

<210> 16
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<220>
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 <223> ZFP

<220>
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 <222> (131)..(140)
 <223> ZC linker

<220>
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 <222> (141)..(336)
 <223> FokI cleavage half-domain

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Ala Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe
 20 25 30

Ser Arg Ser Asp Asn Leu Ser Glu His Ile Arg Thr His Thr Gly Glu
 35 40 45

Lys Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Arg Asn Ala
 50 55 60

His Arg Ile Asn His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe
 65 70 75 80

Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Thr Leu Ser
 85 90 95

Glu His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile
 100 105 110

Cys Gly Arg Lys Phe Ala Ala Arg Ser Thr Arg Thr Thr His Thr Lys
 115 120 125

Ile His Leu Arg Gln Lys Asp Ala Ala Arg Gly Ser Gln Leu Val Lys
 130 135 140

Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys Leu Lys Tyr
 145 150 155 160

Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg Asn Ser Thr
 165 170 175

Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe Met Lys Val
 180 185 190

Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser Arg Lys Pro Asp Gly
 195 200 205

Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val Ile Val Asp
 210 215 220

Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly Gln Ala Asp
 225 230 235 240

Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr Arg Asn Lys His Ile
 245 250 255

Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val Thr Glu Phe
 260 265 270

Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr Lys Ala Gln
 275 280 285

Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn Gly Ala Val Leu Ser
 290 295 300

Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala Gly Thr Leu
 305 310 315 320

Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn Gly Glu Ile Asn Phe
 325 330 335

<210> 17
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 <212> PRT
 <213> Artificial

<220>
 <223> 5-10 ZFP/FokI fusion

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<220>
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 <223> FokI cleavage half-domain

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 20 25 30

Ser Arg Ser Asp Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu
 35 40 45

Lys Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Ser Ser
 50 55 60

Asn Arg Lys Thr His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln
 65 70 75 80

Lys Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp
 85 90 95

Ser Leu Ser Val His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala
 100 105 110

Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asn Arg Ile Thr
 115 120 125

His Thr Lys Ile His Leu Arg Gln Lys Asp Ala Ala Arg Gly Ser Gln
 130 135 140

Leu Val Lys Ser Glu Leu Glu Glu Lys Lys Ser Glu Leu Arg His Lys
 145 150 155 160

Leu Lys Tyr Val Pro His Glu Tyr Ile Glu Leu Ile Glu Ile Ala Arg
 165 170 175

Asn Ser Thr Gln Asp Arg Ile Leu Glu Met Lys Val Met Glu Phe Phe
 180 185 190

Met Lys Val Tyr Gly Tyr Arg Gly Lys His Leu Gly Gly Ser Arg Lys
 195 200 205

Pro Asp Gly Ala Ile Tyr Thr Val Gly Ser Pro Ile Asp Tyr Gly Val
 210 215 220

Ile Val Asp Thr Lys Ala Tyr Ser Gly Gly Tyr Asn Leu Pro Ile Gly
 225 230 235 240

Gln Ala Asp Glu Met Gln Arg Tyr Val Glu Glu Asn Gln Thr Arg Asn
 245 250 255

Lys His Ile Asn Pro Asn Glu Trp Trp Lys Val Tyr Pro Ser Ser Val
 260 265 270

Thr Glu Phe Lys Phe Leu Phe Val Ser Gly His Phe Lys Gly Asn Tyr
 275 280 285

Lys Ala Gln Leu Thr Arg Leu Asn His Ile Thr Asn Cys Asn Gly Ala
 290 295 300

Val Leu Ser Val Glu Glu Leu Leu Ile Gly Gly Glu Met Ile Lys Ala
 305 310 315 320

Gly Thr Leu Thr Leu Glu Glu Val Arg Arg Lys Phe Asn Asn Gly Glu
 325 330 335

Ile Asn Phe

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 <213> Artificial

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 aacggccaca agttcagcgt gtccggcgag ggcgagggcg atgccaccta cggcaagctg 180
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 gtgaacttca agatccgccca caacatcgag gacggcagcg tgcagctcgc cgaccactac 600
 cagcagaaca cccccatcgg cgacggcccc gtgctgctgc ccgacaacca ctacctgagc 660
 acccagtcgg ccctgagcaa agaccccaac gagaagcgcg atcacatggt cctgctggag 720
 ttcgtgaccg ccgccgggat cactctcggc atggacgagc tgtacaagta aagcggccgc 780
 gactctagat cataatc 797

<210> 19
 <211> 795
 <212> DNA
 <213> Artificial

<220>
 <223> mutant defective eGFP gene

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 aagggcgagg agctgttcac cgggggtggtg cccatcctgg tcgagctgga cggcgacgta 120
 aacggccaca agttcagcgt gtccggcgag ggcgagggcg atgccaccta cggcaagctg 180
 accctgaagt tcatctgcac caccggcaag ctgcccgtgc cctggcccac cctcgtgacc 240
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cttcaagtcc gccatgcccc aaggctacgt ccaggagcgc accatcttct tcaaggacga	360
cggcaactac aagacccgcg ccgaggtgaa gtctgagggc gacaccctgg tgaaccgcat	420
cgagctgaag ggcacgcact tcaaggagga cggcaacatc ctggggcaca agctggagta	480
caactacaac agccacaacg tctatatcat ggccgacaag cagaagaacg gcatcaaggt	540
gaacttcaag atccgccaca acatcgagga cggcagcgtg cagctcgccg accactacca	600
gcagaacacc cccatcggcg acggccccgt gctgctgccc gacaaccact acctgagcac	660
ccagtcgcgc ctgagcaaag accccaacga gaagcgcgat cacatggtcc tgctggagtt	720
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ctctagatca taatc	795

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ctgaagttca tctgcaccac cggcaagctg cccgtgccct ggccccaccct cgtgaccacc	180
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gagctgaagg gcatcgactt caaggaggac ggcaacatcc tggggcaca gctggagtac	420
aactacaaca gccacaacgt ctatatcatg gccgacaagc agaagaacgg catcaagggtg	480
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cagaacaccc ccatcggcga cggccccgtg ctgctgcccg acaaccacta cctgagcacc	600
cagtccgccc tgagcaaaga cccaacgag aagcgcgatc acatggtcct gctggagttc	660
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tctagatcat aatc	734

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<220>

<223> eGFP insert in pCR(R)4-TOPO

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gagctgaagg	gcacgcactt	caaggaggac	ggcaacatcc	tggggcacia	gctggagtag	420
aactacaaca	gccacaacgt	ctatatcatg	gccgacaagc	agaagaacgg	catcaagggtg	480
aacttcaaga	tccgccacia	catcgaggac	ggcagcgtgc	agctcgccga	ccactaccag	540
cagaacaccc	ccatcggcga	cggccccgtg	ctgctgcccg	acaaccacta	cctgagcacc	600
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agtctagagg	gcccgtttta	acccgctgat	cagcctcgac	tgtgccttct	agttgccagc	780
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<210> 22

<211> 116

<212> PRT

<213> Artificial

<220>

<223> sca-29b

<400> 22

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
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Gln Ser Gly Asp Leu Thr Arg His Ile Arg Thr His Thr Gly Glu Lys
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Thr Ser Ala Asn
35 40 45

Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln Lys
50 55 60

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala
65 70 75 80

Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys
85 90 95

Asp Ile Cys Gly Arg Lys Phe Ala Gln Ser Gly His Leu Ser Arg His
100 105 110

Thr Lys Ile His
115

<210> 23

<211> 113

<212> PRT

<213> Artificial

<220>

<223> sca-36a

<400> 23

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
1 5 10 15

Arg Ser Gln Thr Arg Lys Thr His Ile Arg Thr His Thr Gly Glu Lys
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Gln Lys Arg Asn
35 40 45

Arg Thr Lys His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln

50	55	60
Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala Leu Ser Arg		
65	70	75 80
His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys		
	85 90	95
Gly Arg Lys Phe Ala Gln Ser Gly Asn Leu Ala Arg His Thr Lys Ile		
	100 105	110

His

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 <212> PRT
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<220>
 <223> sca-36b

<400> 24

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Thr Ser Gly Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys	
20 25 30	
Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asp	
35 40 45	
Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln Lys	
50 55 60	
Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala	
65 70 75 80	
Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys	
85 90 95	
Asp Ile Cys Gly Arg Lys Phe Ala Gln Ser Gly Asn Leu Ala Arg His	
100 105 110	
Thr Lys Ile His	
115	

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<212> PRT
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<220>
<223> sca-36c

<400> 25

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
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Thr Ser Ser Ser Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Asp Arg Ser Asp
35 40 45

Leu Ser Arg His Thr Lys Ile His Thr Gly Gly Gly Gly Ser Gln Lys
50 55 60

Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser Asp Arg Ser Ala
65 70 75 80

Leu Ser Arg His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys
85 90 95

Asp Ile Cys Gly Arg Lys Phe Ala Gln Ser Gly Asn Leu Ala Arg His
100 105 110

Thr Lys Ile His
115

<210> 26
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<220>
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<400> 26

His Gln Arg Thr His Gln Asn Lys Lys Gln Leu Val
1 5 10

<210> 27
<211> 12
<212> DNA
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<220>
<223> hSMC1L1 Gene target sequence

<400> 27
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12

<210> 28
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<212> PRT
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<220>
<223> hSMC1L1 Gene F1

<400> 28

Arg Ser His Asp Leu Ile Glu
1 5

<210> 29
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<212> PRT
<213> Artificial

<220>
<223> hSMC1L1 Gene F2

<400> 29

Thr Ser Ser Ser Leu Ser Arg
1 5

<210> 30
<211> 7
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Arg Ser Asp Ser Leu Ser Ala
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<210> 87
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 Arg Ser Asp Asp Leu Ser Lys
 1 5

<210> 88
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Asp Asn Ser Asn Arg Ile Lys
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Leu Val

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Gln Ser Gly Ser Leu Thr Arg
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Gln Ser Gly Asp Leu Thr Arg
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<223> 296S F1

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Gln Ser Gly Ser Leu Thr Arg
1 5

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<223> 296S F2

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Gln Ser Gly Asp Leu Thr Arg
1 5

<210> 143

<211> 7

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<223> 296S F3

<400> 143

Gln Ser Gly Asn Leu Ala Arg
1 5

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 ccatgtgatac gcgcttctc 19

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 <400> 148
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<210> 154

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1 5

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<220>
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<400> 155

Arg Ser Asp Thr Leu Ser Glu
1 5

<210> 156
<211> 7
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<220>
<223> IL2Rgamma Gene F4

<400> 156

Ala Arg Ser Thr Arg Thr Asn
1 5

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12

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Arg Ser Asp Thr Leu Ser Glu
1 5

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<400> 159

Ala Arg Ser Thr Arg Thr Thr
1 5

<210> 160

<211> 7

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Arg Ser Asp Ser Leu Ser Lys
1 5

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<211> 7

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Gln Arg Ser Asn Leu Lys Val
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<211> 117

<212> PRT

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1 5 10 15

Arg Ser Asp Asn Leu Ser Val His Ile Arg Thr His Thr Gly Glu Lys
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Arg Asn Ala His
35 40 45

Arg Ile Asn His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln
50 55 60

Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Thr Leu Ser Glu
65 70 75 80

His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys
85 90 95

Gly Arg Lys Phe Ala Ala Arg Ser Thr Arg Thr Asn His Thr Lys Ile
100 105 110

His Leu Arg Gly Ser
115

<210> 163
<211> 117
<212> PRT
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<220>
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<400> 163

Met Ala Glu Arg Pro Phe Gln Cys Arg Ile Cys Met Arg Asn Phe Ser
1 5 10 15

Arg Ser Asp Thr Leu Ser Glu His Ile Arg Thr His Thr Gly Glu Lys
20 25 30

Pro Phe Ala Cys Asp Ile Cys Gly Arg Lys Phe Ala Ala Arg Ser Thr
35 40 45

Arg Thr Thr His Thr Lys Ile His Thr Gly Ser Gln Lys Pro Phe Gln
50 55 60

Cys Arg Ile Cys Met Arg Asn Phe Ser Arg Ser Asp Ser Leu Ser Lys
65 70 75 80

His Ile Arg Thr His Thr Gly Glu Lys Pro Phe Ala Cys Asp Ile Cys
85 90 95

Gly Arg Lys Phe Ala Gln Arg Ser Asn Leu Lys Val His Thr Lys Ile
100 105 110

His Leu Arg Gly Ser
115

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<223> IL-2Rgamma insert sequence

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Phe Arg Val Arg Ser Arg Phe Asn Pro Leu Cys Gly Ser
1 5 10

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<211> 39
<212> DNA
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<220>
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<400> 166
gattcaacca gacagataga agg

23

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<220>
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22

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 <220>
 <223> Exon 5 reverse primer

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 ttccttccat caccaaaccc tcttg 25

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 <210> 171
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 <223> ex5_1.5detR3

 <400> 171
 ttccttccat caccaaaccc tcttg 25

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 <400> 176
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 <400> 177
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 gaagtctgcc gt 12

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 gaagtctgcc gtt 13

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 <223> sca-36c
 <400> 180
 gaagtctgcc gtt 13

<210> 181
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 <212> DNA
 <213> Artificial
 <220>
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 <223> sca-r29b
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 acgtagactg agg 13

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<400> 183

Thr Gly Gly Gly Gly Ser Gln Lys Pro
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Gln Ser Gly Asp Leu Thr Arg
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Thr Ser Ala Asn Leu Ser Arg
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<400> 186

Asp Arg Ser Ala Leu Ser Arg
1 5

<210> 187
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Gln Ser Gly His Leu Ser Arg
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Arg Ser Gln Thr Arg Lys Thr
1 5

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Gln Lys Arg Asn Arg Thr Lys
1 5

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Asp Arg Ser Ala Leu Ser Arg
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Thr Ser Gly Ser Leu Ser Arg
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Asp Arg Ser Asp Leu Ser Arg
1 5

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Asp Arg Ser Ala Leu Ser Arg
1 5

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Gln Ser Gly Asn Leu Ala Arg
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 Thr Ser Ser Ser Leu Ser Arg
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 Asp Arg Ser Asp Leu Ser Arg
 1 5

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 Asp Arg Ser Ala Leu Ser Arg
 1 5

 <210> 199
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 <220>
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 Gln Ser Gly Asn Leu Ala Arg
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Xaa Xaa Xaa Xaa His Xaa Xaa Xaa Xaa Xaa His
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<400> 202
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<210> 203
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<220>
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<400> 203
aactcggata at

12

<210> 204
<211> 7
<212> PRT
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<220>
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<400> 204

Asp Arg Ser Thr Leu Ile Glu
1 5

<210> 205
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<220>
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Ser Ser Ser Asn Ser Leu Arg
1 5

<210> 206
<211> 7
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<220>
<223> IL2-1 zinc finger F3

<400> 206

Arg Ser Asp Asp Leu Ser Lys
1 5

<210> 207
<211> 7
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<220>

<223> IL2-1 zinc finger F4

<400> 207

Asp Asn Ser Asn Arg Ile Lys
1 5

<210> 208

<211> 25

<212> DNA

<213> Artificial

<220>

<223> PCR primer

<400> 208

ggatcccaac tagtcaaaag tgaac

25

<210> 209

<211> 25

<212> DNA

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<220>

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<400> 209

ctcgagttaa aagtttatct cgccg

25

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<211> 7

<212> PRT

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<223> ZC linker

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Pro Lys Lys Lys Arg Lys Val

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32